



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :  C12N 5/04, 15/09, 15/11, 15/52, A01H 5/00, 5/10		A1	(11) International Publication Number: <b>WO 00/11138</b>
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(21) International Application Number: PCT/US99/20849		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 24 August 1999 (24.08.99)			
(30) Priority Data: 60/097,684 24 August 1998 (24.08.98) US			
(71) Applicant (for all designated States except US): RUTGERS, THE STATE UNIVERSITY OF NEW JERSEY [US/US]; Old Queens, Somerset Street, New Brunswick, NJ 08903 (US).			
(72) Inventors; and		Published	
(75) Inventors/Applicants (for US only): CHEN, Tseh, An [US/US]; 55 Juniper Way, Basking Ridge, NJ 07920 (US). CHEN, Shou-Yi [CN/CN]; Lab Plant Technology, Lab 803, Institute of Genetics, Chinese Academy of Science, Beijing 100101 (CN). ZHANG, Geng-Yun [CN/US]; 78 Apt. 1A Chester Circle, New Brunswick, NJ (US). BELANGER, Faith, C. [US/US]; 40 Ross Hall Boulevard North, Piscataway, NJ (US).		With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.	
(74) Agents: KLANN, Ellen, M. et al.; Dann, Dorfman, Herrell and Skillman, Suite 720, 1601 Market Street, Philadelphia, PA 19103 (US).			
(54) Title: SALT-TOLERANT TRANSGENIC TURFGRASS			
(57) Abstract			
<p>A transgenic turfgrass plant expressing a betaine aldehyde dehydrogenase-encoding transgene is provided. The transgenic plant displays significantly increased tolerance to salinity than does its non-transgenic equivalents. The plant also displays increased tolerance to drought conditions. The salt- and drought-tolerant transgenic turfgrass may be planted in regions of high salinity, such as seaside, or in regions where irrigation water is scarce.</p>			

## PATENT COOPERATION TREATY

PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

REC'D 25 JAN 2001

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(PCT Article 36 and Rule 70)

Applicant's or agent's file reference RUT 98-0068P	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US99/20849	International filing date (day/month/year) 24 AUGUST 1999	Priority date (day/month/year) 24 AUGUST 1998
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant RUTGERS, THE STATE UNIVERSITY OF NEW JERSEY		

<ol style="list-style-type: none"> <li>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</li> <li>2. This REPORT consists of a total of <u>4</u> sheets.</li> </ol> <p><input type="checkbox"/> This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of <u>0</u> sheets.</p>
<ol style="list-style-type: none"> <li>3. This report contains indications relating to the following items:           <ul style="list-style-type: none"> <li>I <input checked="" type="checkbox"/> Basis of the report</li> <li>II <input type="checkbox"/> Priority</li> <li>III <input type="checkbox"/> Non-establishment of report with regard to novelty, inventive step or industrial applicability</li> <li>IV <input type="checkbox"/> Lack of unity of invention</li> <li>V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</li> <li>VI <input type="checkbox"/> Certain documents cited</li> <li>VII <input type="checkbox"/> Certain defects in the international application</li> <li>VIII <input type="checkbox"/> Certain observations on the international application</li> </ul> </li> </ol>

Date of submission of the demand 15 MARCH 2000	Date of completion of this report 13 DECEMBER 2000
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer  MELISSA KIMBALL Telephone No. (703) 308-0196

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US99/20849

## I. Basis of the report

## 1. With regard to the elements of the international application: \*

 the international application as originally filed the description:pages 1-28, as originally filed  
pages NONE  
pages NONE, filed with the demand the claims:pages 29-32, as originally filed  
pages NONE, as amended (together with any statement) under Article 19  
pages NONE, filed with the demand  
pages NONE, filed with the letter of the drawings:pages 1-2, as originally filed  
pages NONE, filed with the demand  
pages NONE, filed with the letter of the sequence listing part of the description:pages NONE, as originally filed  
pages NONE, filed with the demand  
pages NONE, filed with the letter of

## 2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language \_\_\_\_\_ which is:

- the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- the language of publication of the international application (under Rule 48.3(b)).
- the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

## 3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- contained in the international application in printed form.
- filed together with the international application in computer readable form.
- furnished subsequently to this Authority in written form.
- furnished subsequently to this Authority in computer readable form.
- The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4.  The amendments have resulted in the cancellation of:

- the description, pages NONE
- the claims, Nos. NONE
- the drawings, sheets/fig NONE

5.  This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).\*\*

\* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

\*\*Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US99/20849

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement****1. statement**

Novelty (N)	Claims <u>1-25</u>	YES
	Claims <u>NONE</u>	NO
Inventive Step (IS)	Claims <u>NONE</u>	YES
	Claims <u>1-25</u>	NO
Industrial Applicability (IA)	Claims <u>1-25</u>	YES
	Claims <u>NONE</u>	NO

**2. citations and explanations (Rule 70.7)**

Claims 1-25 lack an inventive step under PCT Article 33(3) as being obvious over Nehra et al. in view of Adams et al.

The claims are drawn to transgenic Graminaceous cells, plants and seeds comprising transgenes encoding enzymes involved in the glycine betaine biosynthetic pathway, or more specifically, encoding betaine aldehyde dehydrogenase (BADH).

Nehra et al. teach transgenic Graminaceous plants and cells and methods of creating them (col. 3, for example). They teach expressing foreign genes in monocots such as stress tolerance genes including betaine aldehyde dehydrogenase (col. 7, line 5-50).

Nehra et al. do not teach specific plasmids or types of turfgrass for use in the creation of stress tolerant plants expressing BADH.

Adams et al. teach that stress tolerance in transgenic monocots is imparted through the expression of foreign osmoprotectants or over expression of native ones such as glycine-betaine (col. 2, line 40-67 and col. 7, lines 1-20). They teach salt resistance, particularly NaCl tolerance, in transformed plants (col. 4, line 43-67). Adams et al. teach expression vectors for use in their methods (col. 8, line 20-26) and methods of introducing transgenes to Graminaceous cells (col. 9-10).

The invention lacks an inventive step because Nehra et al. teach expressing BADH in Graminaceous cells and because Adams et al. further expand on the benefits of transgenic expression of osmoprotectants to produce salt tolerant monocot plants. The selection of the plasmid used and the choice of Graminaceous plant is regarded as the optimization of design parameters.

Claims 1-25 meet the criteria set out in PCT Article 33(2) and (4), because the prior art does not teach transgenic turf grass expressing BADH and such plants, seeds and cells have industrial applicability as stress tolerant plants are useful for ornamental (Continued on Supplemental Sheet.)

**Supplemental Box**

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

Sheet 10

**CLASSIFICATION:**

The International Patent Classification (IPC) and/or the National classification are as listed below:

IPC(7): C12N 5/04, 15/09, 15/11, 15/52; A01H 5/00, 5/10 and US Cl.: 435/419, 430, 172.1, 172.3; 800/298, 320

**V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):**  
plantings in environments having salt stress or other stresses.

## ----- NEW CITATIONS -----

NONE

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/20849

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C12N 5/04, 15/09, 15/11, 15/52; A01H 5/00, 5/10  
 US CL :435/419, 430, 172.1, 172.3; 800/298, 320

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/419, 430, 172.1, 172.3; 800/298, 320

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST, DERWENT, CAS ONLINE, AGRICOLA

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,780,709 A (ADAMS et al.) 14 July 1998, col. 2, lines 40-67 and col. 7-8.	1-25
Y	US 5,589,617 A (NEHRA et al.) 31 December 1996, col. 2, lines 55-60, col. 3 and col. 7, lines 9-10 and 37-39.	1-25
T,E	US 5,981,842 A (WU et al.) 09 November 1999, see entire document.	1-25
Y	LILIUS et al. Enhanced NaCl stress tolerance in transgenic tobacco expressing bacterial choline dehydrogenase. Bio/Technology. February 1996, Vol. 14, pages 177-180, see especially page 177, col. 1 and 180, col. 1.	1-25

Further documents are listed in the continuation of Box C.  See patent family annex.

Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Z"	document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

30 NOVEMBER 1999

Date of mailing of the international search report

03 February 2000 (03.02.2000)

Name and mailing address of the ISA/US  
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Authorized officer

MELISSA KIMBALL

Telephone No. (703) 308-0196

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/20849

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NAKAMURA et al. Expression of a betaine aldehyde dehydrogenase gene in rice, a glycinebetaine nonaccumulator, and possible localization of its protein in peroxisomes. The Plant Journal. 1997, Vol. 11, No. 15, pages 1115-1120, especially page 1115, col. 2 and page 1118.	1-25
Y	TROSSAT et al. Transgenically expressed betaine aldehyde dehydrogenase efficiently catalyzes oxidation of dimethylsulfoniopropionaldehyde and w-aminoaldehydes. Plant Physiology. 1997, Vol. 113, pages 1457-1461, see entire document.	1-25
Y	RATHINASABAPATHI et al. Cultivated and wild rices do not accumulate glycinebetaine due to deficiencies in two biosynthetic steps. Crop Science. May-June 1993, Vol. 33, pages 534-538, see entire document.	1-25

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